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Dantrolene Dose Response in Malignant Hyperthermia-susceptible (MHS) Swine:

Method to Obtain Prophylaxis and Therapeusis

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The authors examined the thesis that a dose of dantrolene producing 95 per cent of maximal muscle relaxation (ED_{95}) would effectively prevent or treat malignant hyperthermia (MH). In one group of four pigs sensitive to malignant hyperthermia (MHS) a dose response to intravenous dantrolene was determined by quantitation of toe twitch tension. From these data, the ED_{95} relaxant dose (3.5 mg/kg) was derived. In a second group of four MHS pigs, the ED_{95} dantrolene dose was administered prior to MH challenge with succinylcholine, 2 mg/kg, and halothane, 1.5 per cent. MH was prevented in each animal, while measurements of arterial pressure, arterial blood-gas, *p*H and lactate values, rectal temperature, and heart rate were done. Later, MH rapidly developed in the same four animals when they were again challenged. When treated with the ED_{95} dose, each animal survived. Finally, each pig was challenged without dantrolene prophylaxis or therapeusis, and all succumbed from MH. Previous studies have shown the efficacious use of dantrolene in prevention or treatment of porcine MH, but doses used have varied, without rationale. The present study shows that in MHS pigs the ED_{95} muscle relaxant dose of dantrolene (3.5 mg/kg) successfully prevents and treats MH. (Key words: Hyperthermia: malignant pyrexia. Neuromuscular relaxants: dantrolene.)

DANTROLENE SODIUM, a skeletal muscle relaxant, has been successful in preventing and treating malignant hyperthermia (MH) in swine.[1–3] Doses of dantrolene employed have ranged from 1 to 10 mg /kg, intravenously. A basis for determining efficacious dantrolene dosage in MH has not been established. We have developed a method to quantitate indirectly evoked muscle twitch tension in swine.[4] Using this model, we determined the muscle relaxant dose response for intravenously administered dantrolene in one group of MH-susceptible pigs. We then tested in another group of swine the hypothesis that the derived dantrolene relaxant dose that was 95 per cent effective (ED_{95}) is both prophylactic and therapeutic for a succinylcholine–halothane MH challenge.

Methods and Materials

Four purebred 35–69-kg Poland China swine of both sexes were used to determine dantrolene muscle relaxant response. Each had previously been determined to be MH-susceptible by *in-vitro* caffeine and halothane muscle biopsy contracture responses.[5] Anesthesia was induced and maintained with sodium thiopental administered at a mean dose of 54 ± 9 mg/kg (SE) via an ear-vein catheter. This catheter was used for subsequent fluid and drug administration. After orotracheal intubation the swine breathed spontaneously from a closed-circle carbon dioxide (CO_2) absorption system with an oxygen flow of 0.5 l/min. The exhalation side of the breathing circuit had a respirometer and a pressure gauge. Arterial pressure and blood samples were obtained from a superficial femoral-artery catheter. Electrocardiogram and rectal temperature were recorded.

Foretoe flexion tension, indirectly stimulated, was quantitated as previously described.[4] The baseline tension was 100 g. Dantrolene sodium, 1 mg/ml, was prepared fresh daily by dissolving 1 g of purified powder (Norwich-Eaton), sodium hydroxide, 0.113 g, and mannitol, 43.4 g, in one liter of agitated distilled water at a temperature of 55 C. Prepared in this manner, dantrolene remains in solution for several hours, as the mixture returns to room temperature.

After stabilization of control values, dantrolene, 0.5 mg/kg, was given as an intravenous bolus every 2 min for a cumulative dose of 7.5 mg/kg. Exhaled gas volume was measured during each 2-min period between doses. Peak negative inspiratory effort was obtained by occluding the reservoir bag port and noting the negative pressure generated through the second inspiratory effort. Arterial blood samples for blood-gas and *p*H determinations were drawn 2 min following each 1 mg/kg cumulative dose of dantrolene. Variables were measured 15, 30 and 60 min following the final dose of dantrolene.

Four additional purebred Poland China swine (littermates) of both sexes were used to test the effectiveness of the ED_{95} dose to prevent or treat MH. All had been previously determined to be MH-susceptible by contracture testing. Each pig underwent a series of three succinylcholine–halothane challenges for MH. Anesthesia was induced and maintained with thiopental as in the previous series.

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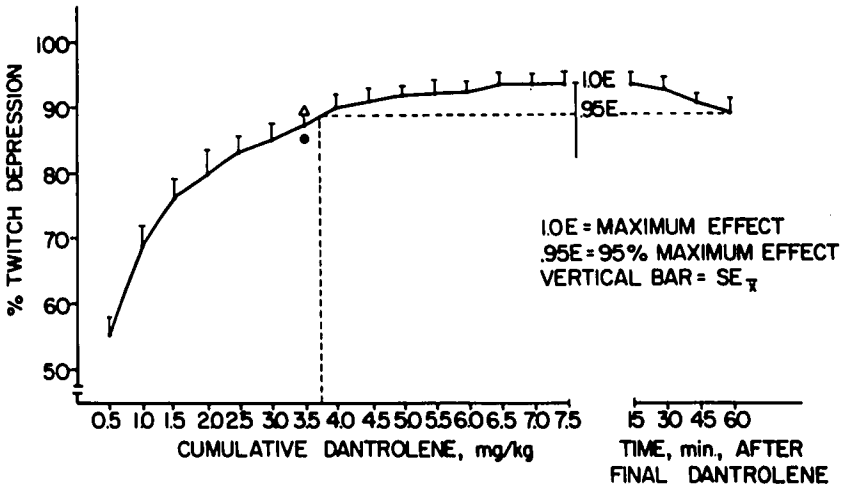

FIG. 1. Dantrolene dose response using foretoe twitch depression in four pigs. Dantrolene, 0.5 mg/kg, given intravenously every 2 min. Derived 95 per cent effective dose was approximately 3.5 mg/kg. Dantrolene, 3.5 mg/kg, bolus intravenous injection, ● = prophylactic and △ = therapeutic dantrolene series pigs (see text).

Oxygen, 4 l/min, was provided to a semiclosed-circle CO_2 absorption system. Respiration was controlled mechanically to maintain a constant peak expired CO_2 tension. Lead II of the electrocardiogram, rectal temperature, foretoe twitch tension, arterial pressure, and arterial blood lactate and blood-gas values (temperature-corrected) were measured as in the previous series. MHS pigs were challenged by bolus succinylcholine, 2 mg/kg, and by simultaneously introducing halothane, 1.5 per cent, into the breathing circuit. Dantrolene solution, 1 mg/ml, was prepared as before. Experiments were carried out in an air-conditioned room at approximately 22 C.

In the first challenge series, dantrolene prophylaxis was tested in the four pigs (49 ± 3 kg) by giving the ED_{95} dantrolene relaxant dose (3.5 mg/kg) as a bolus intravenously. When twitch depression from dantrolene stabilized, the succinylcholine–halothane MH challenge was instituted. After 30 min of exposure to halothane, the vaporizer output was decreased to 1 per cent for an additional 30 min. Sixty minutes after challenge, halothane was discontinued and the pigs were allowed to recover, breathing room air. Experimental variables were measured 15, 30, 45, and 60 min after the challenge was instituted.

In the second challenge series, 14 days later, dantrolene therapeusis was tested. The MHS pigs' weights averaged 51 ± 2 kg. The MH challenge was begun as described above. MH was diagnosed when the following were observed: arterial blood P_{CO_2} exceeding 100 torr, arterial blood *p*H less than 7.0, an increasing rectal temperature, and increasing peak expired CO_2. At this point, halothane was discontinued, and dantrolene, 3.5 mg/kg, was given as a bolus intravenously. Oxygen flow was increased to 10 l/min, and minute ventilation increased in an attempt to lower peak expired CO_2 toward control levels. No other therapy was administered. One hour and 15 min after the challenge, oxygen and mechanical ventilation were discontinued and the pigs were allowed to recover, breathing room air.

In the third and final challenge series, 42 days later, MH was initiated and the variables monitored as before. The pigs' weights averaged 72 ± 4 kg. When the MH syndrome was evident, using the same criteria as in the therapeutic series, halothane was discontinued and hyperventilation initiated in an attempt to lower peak expired CO_2. No other therapy was given.

Statistical evaluation included the Student *t* test for paired data and analysis of variance for grouped

TABLE 1. Cardiopulmonary Variables Assessed during and after Dantrolene Administration, 0.5 mg/kg/2 min*

	Control	Dantrolene 4.0 mg/kg	Dantrolene 7.0 mg/kg	60 Min after Final Dantrolene Dose
Systolic blood pressure (torr)	160 ± 8	168 ± 3	160 ± 5	168 ± 5
Heart rate (/min)	128 ± 18	134 ± 21	131 ± 21	134 ± 24
Pa_{CO_2} (torr)	41 ± 3	41 ± 2	42 ± 5	38 ± 5
pH_a	7.40 ± 0.03	7.44 ± 0.03	7.43 ± 0.05	7.45 ± 0.04
Expired 2-min volume (ml)	280 ± 30	250 ± 20	260 ± 20	280 ± 50
Peak inspiratory effort (cm H_2O)	21 ± 1	17 ± 0.4	17 ± 1	20 ± 1

* Measurements from four pigs were obtained 2 min after indicated doses. None of the variables differed significantly ($P < 0.05$) from the control value. Results are means ± standard errors of the means.

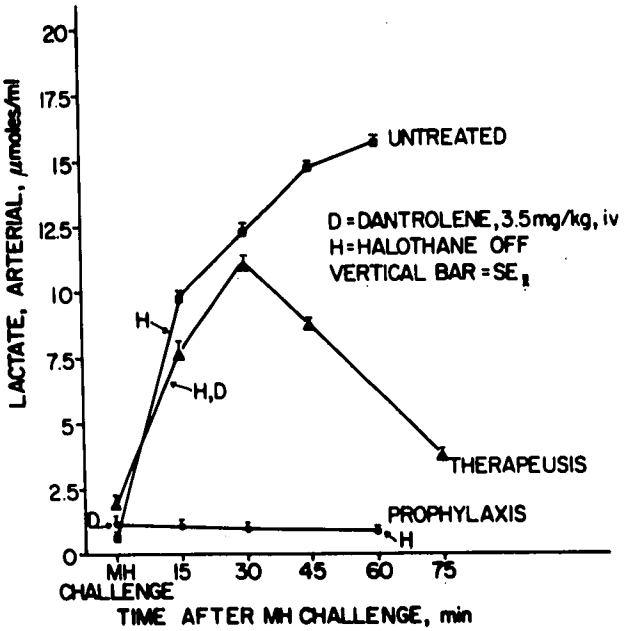

FIG. 2. Arterial blood lactate, μmol/ml, following MH challenge with succinylcholine, 2 mg/kg, and halothane, 1.5 per cent in four pigs. Dantrolene, 3.5 mg/kg, iv, given at *D* for MH prophylaxis or therapeusis. Halothane discontinued at *H*.

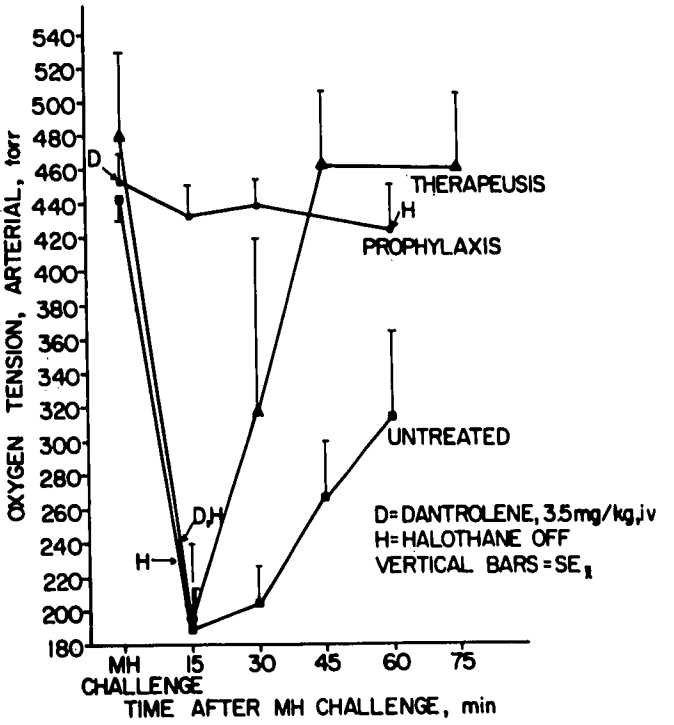

FIG. 3. Arterial blood oxygen tension, torr, following MH challenge with succinylcholine, 2 mg/kg, and halothane, 1.5 percent, in four pigs. Dantrolene, 3.5 mg/kg, iv, given at *D* for MH prophylaxis or therapeusis. Halothane discontinued at *H*.

data. Variances of the means reported within this text are expressed as ± standard errors of the means (±SE). $P < 0.05$ was considered significant.

Results

Maximum twitch depression averaged 93 per cent at a dose range of 5.5–6.5 mg/kg (fig. 1). A plateau in the response was observed in each animal in the first group. The derived 95 per cent effective dose, ED_{95}, was between 3.5 and 4 mg/kg. Dantrolene produced no significant change in cardiopulmonary variables: systolic blood pressure, heart rate, arterial blood P_{CO_2} and *p*H, expired 2-min gas volume, or peak negative inspiratory pressure effort (table 1). An hour following the last dantrolene increment, twitch depression had returned to the ED_{95} level (fig. 1).

In the prophylaxis series of the second group of animals, the ED_{95} dose of dantrolene (3.5 mg/kg) administered just prior to the MH challenge produced a mean twitch depression of 84 ± 4 per cent. This did not differ from the average twitch depression produced in the dose-response group. Immediately following this dose of dantrolene, succinylcholine bolus and 60-min halothane exposure did not produce MH. Arterial blood lactate, *p*H P_{O_2} and P_{CO_2} values changed little; rectal temperature and systolic blood pressure decreased; while heart rate

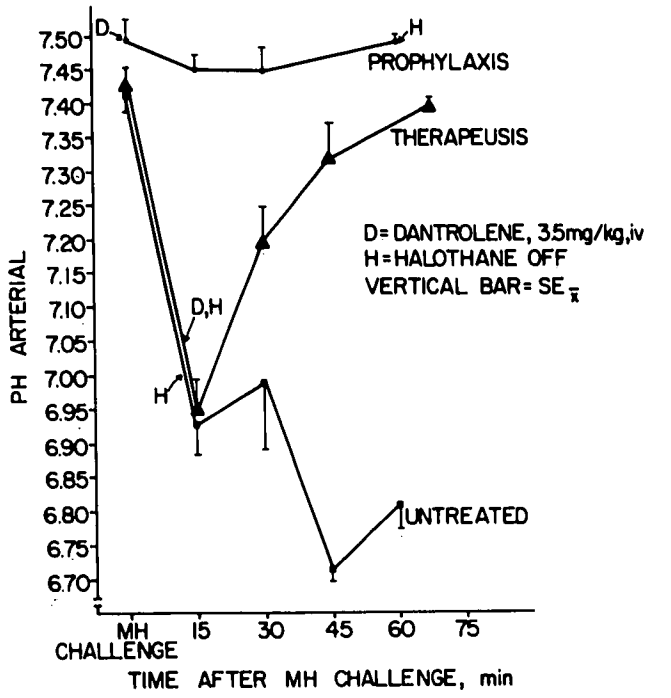

FIG. 4. Arterial blood *p*H following MH challenge with succinylcholine, 2 mg/kg, and halothane, 1.5 per cent, in four pigs. Dantrolene, 3.5 mg/kg, iv, given at *D* for MH prophylaxis or therapeusis. Halothane discontinued at *H*.

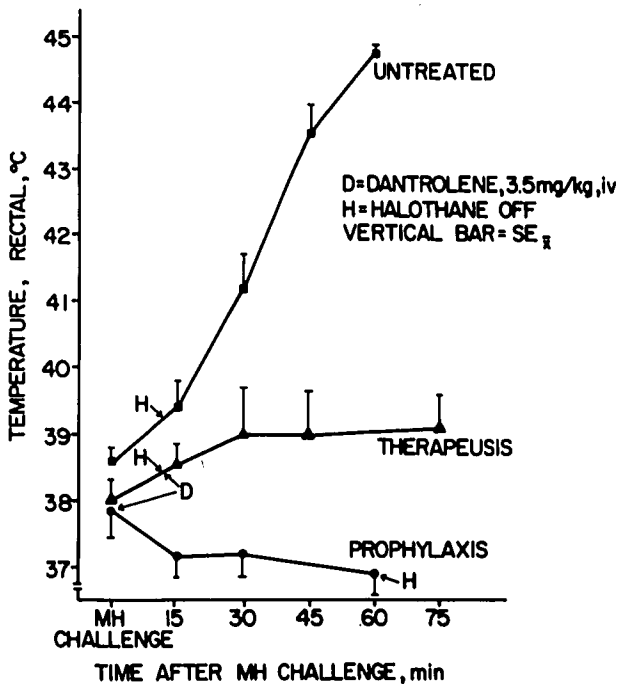

FIG. 5. Temperature, rectal, C, following MH challenge with succinylcholine, 2 mg/kg and halothane, 1.5 per cent, in four pigs. Dantrolene, 3.5 mg/kg, iv, given at *D* for MH prophylaxis or therapeusis. Halothane discontinued at *H*.

increased (figs. 2–5, table 2). Each animal recovered from this experiment without difficulty.

In the therapeutic series of the second group of animals, MH was diagnosed 13 min (mean, range 9–15 min) after challenge. For ease of illustration, the averages of monitored values at the time of diagnosis are reported as the 15-min values (figs. 2–5, table 2). When MH was diagnosed, arterial blood lactate and P_{CO_2} values, rectal temperature, heart rate, and systolic blood pressure were significantly increased while arterial blood *p*H and P_{O_2} values had significantly decreased (figs. 2–5, table 2). The ED_{95} dose of dantrolene (3.5 mg/kg) produced 89 ± 1 per cent twitch depression. This did not differ from the twitch depression observed in the first group (fig. 1). Dantrolene, with cessation of halothane and hyperventilation, effectively reversed the MH episode in each animal (figs. 2–5, table 2). Each animal had an uneventful recovery.

In all pigs in the second group not treated with dantrolene, MH developed within 13 min (mean; range 12–16 min) of challenge. Average monitored values at the time of diagnosis are reported as 15-min values. The severity of the MH episode was comparable to that of the therapeutic series prior to dantrolene, as evidenced by no difference between these groups for arterial blood lactate, P_{O_2} or *p*H values or rectal temperatures. All animals had progressive decreases in systolic blood pressure beginning 30 min after challenge, multifocal ventricular arrhythmia, and profound hyperthermia. Hyperventilation attempts were partially successful in correcting the arterial blood-gas variables (table 2). Death occurred in all animals within 70 min (mean 61 min).

Discussion

A method to determine the dantrolene muscle relaxant response in swine is described. We have shown that the derived ED_{95} dose (3.5 mg/kg) would

TABLE 2. Results (Mean ± SE) of Dantrolene, 3.5 mg/kg, Prophylaxis, Therapeusis, or Nontreatment of MH in Four Susceptible Swine

	MH Challenge Control	Time after Succinylcholine–Halothane Challenge				
		15 Min	30 Min	45 Min	60 Min	75 Min
Pa_{CO_2} (torr)						
Prophylaxis	38 ± 1	45 ± 0.1*	45 ± 3*		41 ± 2	
Therapeusis	40 ± 2	107 ± 5‡	46 ± 4	38 ± 3		42 ± 3
Untreated	50 ± 4†‡	139 ± 10†‡	182 ± 17†‡	161 ± 8†	104 ± 3†‡	
Heart rate (/min)						
Prophylaxis	104 ± 11	122 ± 13*	115 ± 7		103 ± 4	
Therapeusis	138 ± 5	211 ± 33*	168 ± 7‡	167 ± 11		144 ± 13
Untreated	114 ± 7	294 ± 17†‡	261 ± 11†‡	252 ± 5†	213 ± 10†‡	
Systolic blood pressure (torr)						
Prophylaxis	152 ± 11	137 ± 12*	121 ± 9*		117 ± 6*	
Therapeusis	149 ± 7	195 ± 16‡	149 ± 2‡	146 ± 5		148 ± 3
Untreated	177 ± 11	213 ± 32‡	197 ± 19†‡	91 ± 25†	40 ± 9†‡	

* Prophylaxis series different with time from control values, $P < 0.05$.

† Therapeusis and nontreatment series different for a given time, $P < 0.05$.

‡ Therapeusis or nontreatment series different from prophylaxis series for a given time, $P < 0.05$.

prevent or treat MH produced by succinylcholine and halothane. We are confident that dantrolene prevented and treated MH. Arterial blood lactate has been shown to be an early, sensitive indicator of increase in skeletal muscle metabolism during an episode of MH.[2,6] The lactate control values and changes seen during the untreated episode of MH in this study (fig. 2) are similar to those reported in previous studies. The stability of lactate in the prophylactic series supports our conclusion that MH was prevented. The difference between lactate results in the therapeutic and untreated series supports the efficacy of dantrolene with established MH. Arterial blood P_{O_2} responses support our conclusion (fig. 3). Oxygen tension did not change significantly in the prophylaxis series and promptly returned to control with dantrolene therapy, compared with the untreated-MH episode. Rectal temperature responses to MH challenge (fig. 5) were also indicative of the effectiveness of dantrolene. Temperature in the prophylaxis series decreased, while in the therapeutic series it increased, then stabilized. In contrast, untreated animals experienced a profound hyperthermic response to MH challenge.

Dantrolene, given intravenously, has been shown to be efficacious in the prophylaxis or therapeusis of porcine MH in three previous studies.[1–3] Effective doses ranged from 1 to 10 mg/kg. Among these investigations, pig strain, basal anesthesia, technique of MH challenge, and adjunctive therapy differed. In none of these reports was the muscle relaxant effect of dantrolene quantitated. Nor was there unanimity on a minimally effective dose of dantrolene in preventing or treating MH. Harrison investigated Landrace swine using ketamine, thiopental, and nitrous oxide anesthesia.[1] Halothane, 2.5 per cent alone, initiated the MH syndrome. Under these conditions, as little as 1 mg/kg dantrolene was successful in treating MH in one animal, but was unsuccessful in another. For prophylaxis, dantrolene, 3 mg/kg, was successful on two occasions in the same animal. Gronert *et al.*[2] investigated Poland China swine using thiopental and nitrous oxide anesthesia. Halothane, 1 per cent, and succinylcholine, 3 mg/kg, iv, provided the MH challenge. When evaluating MH prophylaxis, dantrolene, 3 mg/kg, was unsuccessful (one animal), but 5 mg/kg was successful (one animal) in preventing detectable signs of MH. Dantrolene, 7.5 mg/kg, bicarbonate, active cooling, and cessation of halothane administration were a successful therapeutic regimen for five animals. A smaller therapeutic dose was not evaluated under these conditions, but dantrolene alone, 5 mg/kg, did not prevent the death of one animal when halothane was not discontinued. Hall *et al.*[3] investigated Pietrain swine using thiopental–nitrous oxide basal anesthesia. The MH challenge was succinylcholine, 50 mg, iv, repeated in 15 min. When the MH syndrome was well established, dantrolene, 500 mg (7.5 mg/kg), was infused over a 30-min period. Five of six swine survived; one died after receiving dantrolene, 3 mg/kg. The results of our investigation are not contradictory to these previous reports, and all investigations lend support to our proposed hypothesis. Cardiopulmonary depression from dantrolene was not reported in these studies.

Dantrolene dose-response results (fig. 1) suggest that if a plateau depressant response is achieved, then the animal is protected against MH for at least an hour until twitch depression recedes to the ED_{95} level. A minimal dantrolene prophylactic dose was not determined, but must be less than 3.5 mg/kg, as twitch depression must have abated during the 60-min halothane exposure.

Cardiopulmonary depression was not observed at any level of dantrolene paralysis in this study (table 1). Dantrolene, in maximum relaxant doses, has not produced cardiopulmonary depression in anesthetized dogs[7,8] or unanesthetized sheep.[7] Thus, it appears that dantrolene can be utilized in anesthetized swine without fear of significant acute morbidity, even when given in a dose (7.5 mg/kg, table 1) more than twice that needed to prevent or treat MH.

We believe that these results in swine might be applied, cautiously, to man. Orally administered dantrolene has been shown to be prophylactic for MH in swine[9,10] and has been recommended for use in man.[11,12] A lyophilized therapeutic intravenous dantrolene preparation† is currently undergoing a clinical trial in North America. Current human dose recommendations for dantrolene for prevention or treatment of MH are empirical. Confirmation of our results in MH-susceptible human subjects would be impossible on ethical grounds. The dantrolene muscle relaxant dose response has not been determined in man, but when known, could be used to adjust MH dantrolene dose recommendations for human subjects.

We conclude that intravenously administered dantrolene, in a dose sufficient to produce maximum indirectly evoked twitch depression, can prevent or treat porcine malignant hyperthermia. Even when dantrolene is utilized prophylactically, only those

† Norwich-Eaton Pharmaceuticals, Norwich, New York.

drugs recognized as safe for MH-susceptible subjects should be employed for anesthesia.

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